

Claims

1. A non-antibody protein comprising a domain having an immunoglobulin-like fold, said non-antibody protein deriving from a reference protein by having a mutated amino acid sequence wherein said non-antibody protein binds with a Kd at least as tight as 1  $\mu$ M to a compound that is not bound as tightly by said reference protein.

2. The derivative protein of claim 1, said derivative protein binding with a Kd at least as tight as 500 nM.

3. The derivative protein of claim 2, said derivative protein binding with a Kd at least as tight as 100 nM.

10 4. The derivative protein of claim 3, said derivative protein binding with a Kd at least as tight as 10 nM.

5. The derivative protein of claim 4, said derivative protein binding with Kd at least as tight as 1 nM.

15 6. The derivative protein of claim 5, said derivative protein binding with a Kd at least as tight as 500 pM.

7. The derivative protein of claim 6, said derivative protein binding with a Kd at least as tight as 100 pM.

8. The derivative protein of claim 7, said derivative protein binding with a Kd at least as tight as 20 pM.

9. The derivative protein of claim 1, wherein said derivative protein contains one, two, or three mutated loops and wherein at least one of said loops contributes to the binding of said derivative protein to said compound.

10. The derivative protein of claim 9, wherein at least two of said loops 5 contribute to said binding of said derivative protein to said compound.

11. The derivative protein of claim 9, wherein three of said loops contribute to said binding of said derivative protein to said compound.

12. The derivative protein of claim 1, wherein said reference protein lacks disulfide bonds.

10. 13. The derivative protein of claim 12, wherein said derivative protein has at least one disulfide bond.

14. The derivative protein of claim 1, wherein said domain having an immunoglobulin-like fold has a molecular mass less than 10 kD.

15. The derivative protein of claim 1, wherein said domain having an 15 immunoglobulin-like fold has a molecular mass greater than 7.5 kD.

16. The derivative protein of claim 1, wherein said domain having an immunoglobulin-like fold has a molecular mass between 7.5-10 kD.

17. The derivative protein of claim 1, wherein said derivative protein is a monomer under physiological conditions.

18. The derivative protein of claim 1, wherein said derivative protein is a dimer under physiological conditions.

19. The derivative protein of claim 1, wherein said reference protein is a naturally-occurring mammalian protein.

5           20. The derivative protein of claim 1, wherein said domain having an immunoglobulin-like fold is mutated and comprises up to 34% mutated amino acids as compared to the immunoglobulin-like fold of said reference protein.

21. The derivative protein of claim 1, wherein said domain having an immunoglobulin-like fold consists of approximately 50-150 amino acids.

10           22. The derivative protein of claim 1, wherein said domain having an immunoglobulin-like fold consists of approximately 50 amino acids.

23. The derivative protein of claim 1, said derivative protein being immobilized on a solid support.

15           24. The derivative protein of claim 23, said derivative protein being part of an array immobilized on a solid support.

25. The derivative protein of claim 23, said solid support being a chip or bead.

26. The derivative protein of claim 1, wherein said derivative protein is joined to a heterologous protein, said heterologous protein being unable to bind to said

compound.

27. The derivative protein of claim 26, wherein said heterologous protein is an antibody or an antibody domain.

5        28. The derivative protein of claim 26, wherein said heterologous protein comprises an immunoglobulin F<sub>c</sub> domain.

10        29. The derivative protein of claim 26, wherein said heterologous protein is selected from the group consisting of a complement protein, a toxin protein, and an albumin protein.

15        30. The derivative protein of claim 1, wherein said derivative protein is covalently bound to a nucleic acid.

20        31. The derivative protein of claim 30, wherein said nucleic acid encodes said protein.

25        32. The derivative protein of claim 1, wherein said derivative protein is derived from a fibronectin or fibronectin dimer, tenascin, N-cadherin, E-cadherin, ICAM, titin, GCSF-receptor, cytokine receptor, glycosidase inhibitor, antibiotic chromoprotein, myelin membrane adhesion molecule P0, CD8, CD4, CD2, class I MHC, T-cell antigen receptor, CD1, C2 and I-set domains of VCAM-1, I-set immunoglobulin domain of myosin-binding protein C, I-set immunoglobulin domain of myosin-binding protein H, I-set immunoglobulin domain of telokin, NCAM, twitchin, neuroglian, growth hormone receptor, erythropoietin receptor, prolactin receptor, interferon-gamma receptor,  $\beta$ -galactosidase/glucuronidase,  $\beta$ -glucuronidase, transglutaminase, T-cell antigen receptor,

superoxide dismutase, tissue factor domain, cytochrome F, green fluorescent protein, GroEL, or thaumatin.

33. A nucleic acid encoding the derivative protein of claim 1.

34. A method of obtaining a derivative non-antibody protein which binds to a  
5 compound, said method comprising:

(a) providing a non-antibody scaffold protein comprising an immunoglobulin-like fold, wherein said scaffold protein does not bind to said compound with a  $K_d$  as tight as  $1 \mu\text{M}$ ;

10 (b) generating mutated derivatives of said non-antibody scaffold protein, thereby producing a library of mutated proteins;

(c) contacting said library with said compound;

(d) selecting from said library at least one derivative protein which binds to said compound with a  $K_d$  at least as tight as  $1 \mu\text{M}$ ; and

15 (e) optionally repeating steps (b) - (d) substituting for the non-antibody scaffold protein in repeated step (b) the product from the previous step (d). 2

35. A method for obtaining a non-antibody protein which binds to a compound, said method comprising:

(a) contacting said compound with a candidate protein, said candidate protein being a derivative non-antibody protein comprising a domain having an immunoglobulin-like fold, said non-antibody protein deriving from a reference protein by having a mutated amino acid sequence wherein said non-antibody protein binds with a  $K_d$  at least as tight as  $1 \mu\text{M}$  to a compound that is not bound as tightly by said reference protein, wherein said contacting is carried out under conditions that allow compound-protein complex formation; and 3

(b) obtaining, from said complex, said derivative protein which binds to said compound.

36. A method for obtaining a compound which binds to a non-antibody protein, said non-antibody protein comprising a domain having an immunoglobulin-like fold and deriving from a reference protein by having a mutated amino acid sequence, wherein said non-antibody protein binds with a  $K_d$  at least as tight as  $1 \mu\text{M}$  to a compound that is not bound as tightly by said reference protein, said method comprising:

(a) contacting said derivative protein with a candidate compound, wherein said contacting is carried out under conditions that allow compound-protein complex formation; and

(b) obtaining, from said complex, said compound which binds to said derivative protein.

37. A method for detecting a compound in a sample, said method comprising:

(a) contacting said sample with a non-antibody protein comprising a domain having an immunoglobulin-like fold, said non-antibody protein deriving from a reference protein by having a mutated amino acid sequence wherein said non-antibody protein binds with a  $K_d$  at least as tight as  $1 \mu\text{M}$  to a compound that is not bound as tightly by said reference protein, wherein said contacting is carried out under conditions that allow compound-protein complex formation; and

(b) detecting said complex, thereby detecting said compound in said sample.

38. A non-antibody protein that binds tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) with a  $K_d$  at least as tight as  $1 \mu\text{M}$ , said protein having a sequence that is less than 20% identical to TNF- $\alpha$  receptor.

39. The non-antibody protein of claim 38, wherein said non-antibody protein comprises a mutated fibronectin type III domain and wherein said protein is mutated in the DE, BC, and FG loops.

40. The non-antibody protein of claim 39, wherein said FG loop is the same  
5 length as the wild-type FG loop.

41. The non-antibody protein of claim 38, wherein said protein comprises any one of the sequences of Figure 25 (SEQ ID NOS: 34-140).

42. A nucleic acid encoding the non-antibody protein of claim 38.

43. A loop structure on a protein, said loop comprising any one of the amino acid sequences of Figure 25 (SEQ ID NOS: 34-140).